

Claims:

1. A method of determining the presence of a nucleic acid in a sample comprising the steps of
 - 5 providing a fluorescent entity capable of indicating the presence of the nucleic acid and capable of providing a signal related to the quantity of the nucleic acid, amplifying the nucleic acid through a plurality of amplification cycles in the presence of the fluorescent entity,
 - 10 measuring fluorescence intensity of the fluorescent entity at each of the plurality of amplification cycles to produce a fluorescent value for each cycle related to the quantity of the nucleic acid present at each cycle,
 - obtaining an individual score from each of a plurality of tests, the plurality of tests comprising a Confidence Interval Test and a Signal-to-Noise Ratio Test, and
 - 15 using the scores to ascertain whether the nucleic acid is present in the sample.
2. The method of claim 1 wherein the plurality of tests further comprise a Channel Consistency Test and an Efficiency Test.
- 20 3. The method of claim 2 wherein the plurality of tests further comprise a Function Ordering Test, a Maximum to Baseline Comparison Test, and a Last Rise Test.
- 25 4. The method of claim 3 wherein the individual scores are each corrected with a predetermined correction factor, and wherein the using step comprises generating a *Score*, wherein *Score* comprises the product of each of the corrected individual scores, divided by a predetermined threshold value.
- 30 5. The method of claim 4 wherein the *Score* is generated according to the formula

$$Score = \frac{(T_1^{P_1})(T_2^{P_2}) \dots (T_n^{P_n})}{Threshold}$$

6. The method of claim 3 wherein the using step comprises
generating a *CallValue*, wherein the *CallValue* comprises the sum of the
5 logarithm of each of the individual scores.

7. The method of claim 6 wherein the *CallValue* is generated
according to the formula

10
$$CallValue = \sum P_i \log T_i - \log(Threshold)$$

wherein P_i is a correction factor chosen for each of the Tests, T_i is the score from each of
the tests, and *Threshold* has a value chosen to provide a convenient dividing point
between positive and negative calls.

15 8. The method of claim 7 wherein the sample is called positive if
CallValue > 1 and the sample is called negative if *CallValue* < -1.

9. The method of claim 3, further comprising the steps of
determining whether the sample has a Late-Rise positive signal, and
20 performing additional amplification cycles.

10. The method of claim 1 wherein the plurality of tests further
comprise at least one test selected from the group consisting of a Channel Consistency
Test, an Efficiency Test, a Function Ordering Test, a Maximum to Baseline Comparison
25 Test, and a Last Rise Test.

11. The method of claim 1 wherein the presence of a nucleic acid is
further verified by melting temperature analysis.

30 12. The method of claim 1 wherein the using step comprises

generating a *CallValue*, wherein the *CallValue* comprises the sum of the logarithm of each of the individual score

13. The method of claim 12 wherein the *CallValue* is generated
5 according to the formula

$$CallValue = P_1 \log T_1 + P_2 \log T_2 - \log(Threshold)$$

wherein:

- 10 T_1 is the score from the Signal-to-Noise Ratio Test,
 T_2 is the score from the Confidence Interval Test,
 P_1 is a correction factor for the Signal-to-Noise Ratio Test,
 P_2 is a correction factor for the Confidence Interval Test, and
 $Threshold$ has a value chosen to provide a convenient dividing point
between positive and negative calls.

- 15 14. The method of claim 13 wherein the value of *Threshold* is chosen
to maximize the greatest number of correct positive calls when *CallValue* > 0 and to
maximize the greatest number of correct negative calls when *CallValue* < 0.

- 20 15. The method of claim 13 wherein
 T_1 is calculated according to the formula

and

- 25 T_2 is calculated according to the formula

$$T_2 = \sum (F_j - L(j))^2 / NoiseLevel$$

16. The method of claim 15 wherein
 P_1 is between -6.0 and -4.0,

-27-

P_2 is between 0.5 and 1.0, and

Threshold is between 1.5 and 2.0.

17. A method of determining the presence of a nucleic acid in a
5 sample comprising the steps of
providing a fluorescent entity capable of indicating the presence of the
nucleic acid and capable of providing a signal related to the quantity of the nucleic acid,
amplifying the nucleic acid through a plurality of amplification cycles in
the presence of the fluorescent entity,
10 measuring fluorescence intensity of the fluorescent entity at each of the
plurality of amplification cycles to produce a fluorescent value for each cycle related to
the quantity of the nucleic acid present at each cycle,
obtaining a score from each of a plurality of tests, each of the plurality of
tests using the fluorescence values to generate the score, and
15 using the scores to ascertain whether the nucleic acid is present in the
sample.

18. A device for determining the presence of a nucleic acid in a
sample comprising
20 an instrument for temperature cycling to amplify the nucleic acid,
a fluorimeter for detecting fluorescence during amplification of the
nucleic acid, the fluorescence obtained from a fluorescent entity capable of providing a
signal related to the quantity of the nucleic acid, and
a processor for performing analysis routines, wherein the processor is
25 programmed to obtain a score from each of a plurality of tests, each of the plurality of
tests using fluorescence values measured by the fluorimeter to generate the score, and to
use the scores to ascertain whether the nucleic acid is present in the sample.

19. The device of claim 18 wherein the plurality of tests comprise a
30 Confidence Interval Test and a Signal-to-Noise Ratio Test.

-28-

20. The device of claim 19 wherein the plurality of tests further comprise a Channel Consistency Test and an Efficiency Test.

5 21. The device of claim 20 wherein the plurality of tests further comprise a Function Ordering Test, a Maximum to Baseline Comparison Test, and a Last Rise Test.

10 22. The device of claim 18 wherein the instrument is configured for rapid thermal cycling.

23. The device of claim 22 wherein the instrument employs capillary tubes and hot air control.

15 24. The device of claim 18 provided in a portable container for field use.